UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

December 12, 2005

MEMORANDUM

Subject: Efficacy Review for Selective Micro Clean-Alpha;
EPA Reg. No. 74986-4; DP Barcode: D321559

From: Marcie Wawzysko Tidd, Microbiologist
Product Science Branch
Antimicrobials Division (7510C)

Thru: Nancy Whyte, Acting Team Leader
Product Science Branch
Antimicrobials Division (7510C)

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

To: Emily Mitchell PM 32 / Wanda Henson
Regulatory Management Branch II
Antimicrobials Division (7510C)

Applicant: Selective Micro Technologies, LLC
66 Cherry Hill Drive, Suite 230
Beverly, MA 01915

Formulation from the Label:

Active Ingredient(s)	% by wt.
Sodium Chlorite	30.5%
Inert Ingredients	69.5%
Total	100.0%

I. BACKGROUND

The product, Selective Micro Clean-Alpha, is an Agency-registered (EPA Reg. No. 74986-4) disinfectant (bactericide, tuberculocide, virucide, fungicide, algaecide) and food-contact and non-food-contact sanitizer for use on hard, non-porous surfaces in commercial, institutional, animal care, and hospital or medical environments. The applicant requested an amendment to the registration of this product to add claims for effectiveness as a disinfectant against Feline calicivirus, Hepatitis A virus, Human immunodeficiency virus type 1, Poliovirus type 1, and Rotavirus. All studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant's representative to EPA (dated August 29, 2005), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-35 (Data Matrix), five studies (MRID Nos. 466141-01 through 466141-05), Statements of No Data Confidentiality Claims for all five studies, the last accepted label (dated January 24, 2005), and the proposed label.

II. USE DIRECTIONS

The product is designed for use in disinfecting hard surfaces in medical and veterinary clinics. The proposed label provided the following instructions for preparing a 500 ppm activated use solution: Fill the pouch with 2 L of tap water. Wait at least 6 hours before use to ensure that the solution reaches full strength. Shake gently before use. Before use, verify the concentration using *Selective Micro Chlorine Dioxide Test Strips*. Activate the product prior to the expiration date stamped on the pouch. Use the activated solution within 15 days of activation.

Directions on the proposed label provided the following information regarding preparation and use of the product as a virucide: Clean surfaces before using the product. Apply a 100 ppm use solution using a mop, sponge, or sprayer, or by immersion, or by clean-in-place application. For a 100 ppm solution, use a dilution device or sprayer with a 1:5 dilution (one part solution to four parts water). Treated surfaces must remain wet for 10 minutes.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Virucides

The effectiveness of virucides must be tested using virological techniques that simulate the conditions under which the product is intended for use. For products with intended use on dry, inanimate environmental surfaces, carrier tests that are variations of either the AOAC Use-Dilution Method (for liquid surface disinfectants) or the AOAC Germicidal Spray Products Test (for surface spray disinfectants) must be used to produce virucidal data. The virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of two different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface (petri dish, glass slide, steel

cylinder, etc.) for a specified exposure period at room temperature. The virus must be assayed by an appropriate virological technique testing a minimum of four determinations for each dilution. The protocol for the viral assay must include viral recovery, cytotoxicity controls, and ID-50 values. Test results should be reported as the reduction of the virus titer by the activity of the germicide (ID-50 of the virus control less the ID-50 of the test system) expressed as $\log_{10}$ and calculated by a statistical method. For virucidal data to be acceptable, the product must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. The calculated viral titers must be reported with the test results. Separate studies on two batches of product are required for each virus. These Agency standards are presented in DIS/TSS-7.

IV. SUMMARY OF SUBMITTED STUDIES

1. MRID 466141-01 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Feline Calicivirus" for Selective Micro Clean-Alpha, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – July 2, 2004. Project Number A02219.

This study was conducted against Feline calicivirus (ATCC VR-782; Strain F-9), using Crandal Reese feline kidney cells (CRFK cells; ATCC CCL-94; propagated in-house) as the host system. Two lots (Lot Nos. 3304-1 and 3307-1) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No. SMT01031504.FCAL (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 253 ppm) to the sponsor-provided product (presumably the contents of an envelope of dry ingredients). The product was held at 20°C for approximately 40 hours before testing. This was then diluted further on the day of testing to achieve a ~100 ppm ClO_2 use solution. The stock virus cultures contained a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 20.0°C in a relative humidity of 44% for 20 minutes. For each lot of product, separate dried virus films were treated with an unspecified amount of the use solution for 10 minutes at 20.0°C. After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Eagle's minimal essential medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus counts, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

2. MRID 466141-02 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Hepatitis A virus" for Selective Micro Clean-Alpha, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – July 7, 2004. Project Number A02221.

This study was conducted against Hepatitis A virus (Strain HM-175; obtained from AppTec Laboratory Services, Camden, NJ), using fetal Rhesus monkey kidney cells (FRhK-4 cells; originally obtained from ViroMed Laboratories, Inc; maintained in-house) as the host system. Two lots (Lot Nos. 3304-01 and 3307-01) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No. SMT01031504.HAV (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 253 ppm) to the sponsor-provided product (presumably the contents of an envelope of dry ingredients). The product was held at 20°C for approximately 16 hours before testing. This was then diluted further on the day of testing to achieve a ~100 ppm ClO_2 use solution. The stock virus cultures contained a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 20.0°C in a relative humidity of 48% for 20 minutes. For each lot of product, separate dried virus films were treated with an unspecified amount of the use solution for 10 minutes at 20.0°C. After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, and 2.0 mM L-glutamine. FRhK-4 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The inoculum was allowed to adsorb for 90 minutes at 36-38°C in a humidified atmosphere of 5-7% CO_2. Post-adsorption, the cultures were re-fed with 1.0 mL of the test medium. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 14 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus counts, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

3. MRID 466141-03 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Human Immunodeficiency Virus Type 1" for Selective Micro Clean-Alpha, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – July 2, 2004. Project Number A02218.

This study was conducted against Human immunodeficiency virus type 1 (Strain HTLV-III_B; obtained from Advanced Biotechnologies, Inc., Columbia, MD), using human CD4+ lymphocytes (MT-2 cells; originally obtained from the National Cancer Institute, Frederick, MD; propagated in-house) as the host system. Two lots (Lot Nos. 3304-1 and 3307-1) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No. SMT01031504.HIV (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 253 ppm) to the sponsor-provided product (presumably the contents of an envelope of dry ingredients). The product was held at 20°C for approximately 40 hours before testing. This was then diluted further on the day of testing to achieve a ~100 ppm ClO_2 use

solution. The stock virus culture was adjusted to contain a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 26.0°C for 20 minutes and then incubated at 36-38°C for an additional 30 minutes to increase the level of dryness. For each lot of product, separate dried virus films were treated with 2.0 mL of the use solution for 10 minutes at 22°C. After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in RPMI 1640 supplemented with 15% heat-inactivated fetal bovine serum, 50 µg/mL gentamicin, and 2.0 mM L-glutamine. MT-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 9 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus counts, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

4. MRID 466141-04 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Poliovirus Type 1" for Selective Micro Clean-Alpha, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – July 8, 2004. Project Number A02222.

This study was conducted against Poliovirus type 1 (Strain Brunhilde; ATCC VR-1000), using Vero cells (ATCC CCL-81; propagated in-house) as the host system. Two lots (Lot Nos. 3304-1 and 3307-1) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No. SMT01031504.POL (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 253 ppm) to the sponsor-provided product (presumably the contents of an envelope of dry ingredients). The product was held at 20°C for approximately 16 hours before testing. This was then diluted further on the day of testing to achieve a ~100 ppm ClO_2 use solution. The stock virus cultures contained a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 20.0°C in a relative humidity of 50% for 20 minutes. For each lot of product, separate dried virus films were treated with an unspecified amount of the use solution for 10 minutes at 20°C. After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Eagle's minimal essential medium supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus counts, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

5. MRID 466141-05 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rotavirus" for Selective Micro Clean-Alpha, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – July 6, 2004. Project Number A02220.

This study was conducted against Rotavirus (Strain WA; obtained from the University of Ottawa, Ontario, Canada), using Rhesus monkey kidney cells (MA-104 cells; originally obtained from ViroMed Laboratories, Inc.; maintained in-house) as the host system. Two lots (Lot Nos. 3304-1 and 3307-1) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No. SMT01031504.ROT (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 253 ppm) to the sponsor-provided product (presumably the contents of an envelope of dry ingredients). The product was held at 20°C for approximately 16 hours before testing. This was then diluted further on the day of testing to achieve a ~100 ppm ClO_2 use solution. The stock virus cultures contained a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 20.1°C in a relative humidity of 53% for 20 minutes. For each lot of product, separate dried virus films were treated with an unspecified amount of the use solution for 10 minutes at 20.1°C. After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in serum-free Eagle's minimal essential medium supplemented with 0.5 µg/mL trypsin, 2.0 mM L-glutamine, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. MA-104 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The inoculum was allowed to adsorb for 1 hour at 36-38°C in a humidified atmosphere of 5-7% CO_2. Post-adsorption, 1.0 mL of the test medium was added to each well, and the cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus counts, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

V. RESULTS

MRID Number	Organism	Results			Dried Virus Control ($TCID_{50}$/0.1 mL)
			Lot No. 3304-1	Lot No. 3307-1	
466141-03	Human immuno-deficiency virus type 1	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{4.5}$ $TCID_{50}$/0.2 mL
		10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.2 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	≥ 3.0 $\log_{10}$	≥ 3.0 $\log_{10}$	
466141-01	Feline calicivirus	10^{-1} to 10^{-9} dilutions	Complete inactivation	Complete inactivation	$10^{6.5}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
466141-02	Hepatitis A virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
466141-04	Poliovirus type 1	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
466141-05	Rotavirus	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.5}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

VI. CONCLUSIONS

1. The submitted efficacy data support the use of the product, Selective Micro Clean-Alpha, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 250 ppm hard water and a 5% organic soil load for a contact time of 10 minutes at a 100 ppm ClO_2 dilution:

Feline calicivirus	MRID No. 466141-01
Hepatitis A virus	MRID No. 466141-02

Human immunodeficiency virus type 1	MRID No. 466141-03
Poliovirus type 1	MRID No. 466141-04
Rotavirus	MRID No. 466141-05

Recoverable virus titers of at least 10^4 were achieved. In studies against Human immunodeficiency virus type 1, cytotoxicity was observed in the 10^{-1} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In studies against all other viruses, complete inactivation (no growth) was observed in all dilutions tested.

VII. RECOMMENDATIONS

1. The proposed label claims that the product, Selective Micro Clean-Alpha, is an effective disinfectant on hard, non-porous surfaces against the following microorganisms for a contact time of 10 minutes at a 100 ppm dilution:

Feline calicivirus
Hepatitis A virus
Human immunodeficiency virus type 1
Poliovirus type 1
Rotavirus

These claims are acceptable, as they are supported by data provided by the applicant.

2. The applicant needs to make the following changes to the proposed label, as appropriate:

- On page 2 under the "Special Instructions for Cleaning and Decontaminating Surfaces and Objects . . . " section, change "1G-1" to read "the product" or "Clean-Alpha."

- In accordance with DIS/TSS-15 guidelines, add a statement regarding if, and how, the product should be removed from treated surfaces after the recommended exposure time.